PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/045

A1

(11) International Publication Number: WO 00/06141

(43) International Publication Date: 10 February 2000 (10.02.00)

(21) International Application Number: PCT/US99/17370

(22) International Filing Date: 30 July 1999 (30.07.99)

(30) Priority Data:
60/094,785 31 July 1998 (31.07.98) US

(71) Applicant (*for all designated States except US*): THE BOARD OF REGENTS OF THE UNIVERSITY AND COMMUNITY COLLEGE SYSTEM OF NEVADA, on behalf of the UNIVERSITY OF NEVADA–RENO [US/US]; 1664 North Virginia Street, Reno, NV 89503–1738 (US).

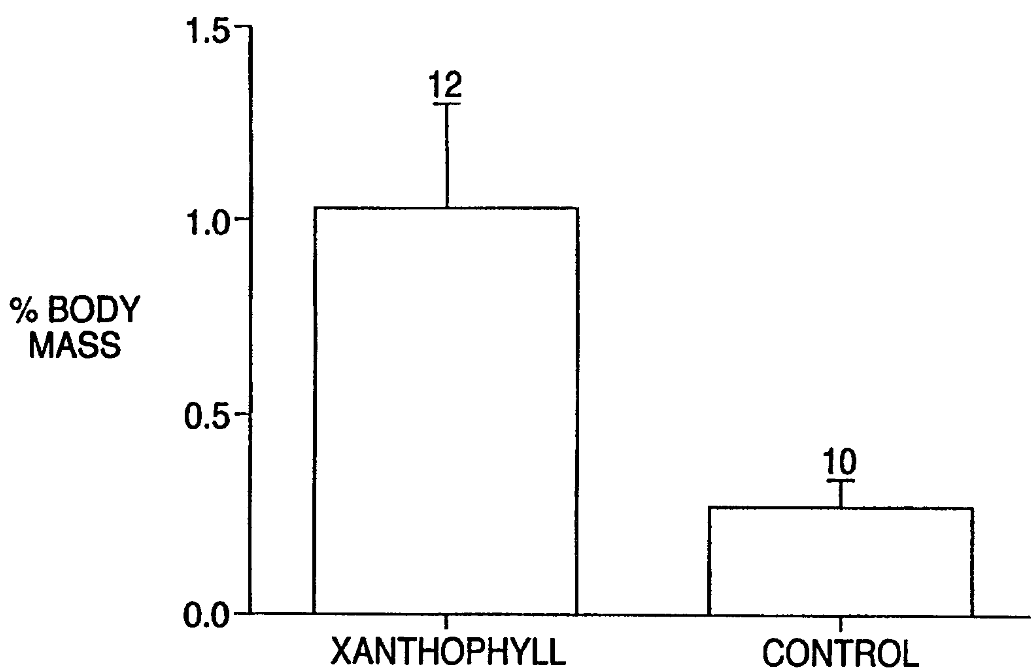
(72) Inventor; and
(75) Inventor/Applicant (*for US only*): DELAHANTY, David, J. [US/US]; 1880 Simpson Avenue, Reno, NV 89503 (US).

(74) Agent: ADLER, Reid, G.; Morgan, Lewis & Bockius LLP, 1800 M Street, N.W., Washington, DC 20036 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: USE OF XANTHOPHYLL TO ACCENTUATE THE EFFECTS OF ESTROGEN



(57) Abstract

The present invention provides methods and compositions comprising xanthophyll for use in accentuating the physiological effects of endogenous (or supplemental) steroids such as estrogens. Specifically, such methods and compositions are useful in enhancing and extending the reproductive capacity of game birds, and enhancing steroid–mediated reproductive responses in animals.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

TITLE: Use of Xanthophyll to Accentuate the Effects of Estrogen

RELATED APPLICATIONS

This application is related to U.S. Provisional Application No. 60/094,785, filed July 31, 1998, which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

The present invention provides methods and compositions for enhancing the reproductive capability of game birds by supplementing their diet with xanthophyll. These methods can be used to increase the number of progeny that can be produced in a single breeding season and to extend the breeding season of game birds. The invention also relates to methods and compositions useful in enhancing or accentuating the effects of steroids, particularly estrogen, in animals.

BACKGROUND OF THE INVENTION

1. The Breeding of Game Birds.

Game bird breeding is practiced worldwide and a global market exists for game bird chows. In the United States alone, hundred of thousands of quail, pheasants, partridge, grouse, wild turkeys and waterfowl are raised on game farms and sold annually to hunting clubs and shooting preserves. The game birds used in these hunting clubs and shooting preserves, while edible, are bred exclusively for the sport of hunting and not for mass consumption, as opposed to domestic poultry (*i.e.*, domestic turkey and chicken), which are bred exclusively for consumption. Game bird chow is sold widely throughout the United States and other countries as well. In addition to the brisk business of game farming, a substantial market exists surrounding recreational birds known as Aviculture. In this market, game birds are bred for their beauty, zoological interest, conservation and market value much as other wild animals such as exotic fish, reptiles and tigers are kept,

bred and sold.

One problem involved in game bird breeding is that game birds breed seasonally as opposed to commercial poultry which breed throughout the year. As a result, game bird breeders must maximize their egg production with a single, short, breeding season in the Spring of each year. Female game birds who start breeding late, who fail to come into breeding condition, and females with low fecundity can ruin a breeder's entire output for that year. Game bird breeders are always looking for ways that allow them to consistently obtain the highest egg production possible from their female game birds in order to maximize profits, because these breeders have incurred significant costs to maintain these birds while waiting for the breeding season to begin. Processes which would accelerate the onset and extend the duration of the breeding condition in female game birds would be beneficial to game bird breeders because it would ensure a consistent output for each breeding season.

Game bird nutrition is poorly understood due to the absence of published scientific research in this field. Current game bird chows are minor variations of domestic poultry chows. Game birds require special nutritional needs during their rapid, annual breeding season. If these nutritional needs are not met, game birds temporarily stop egg laying while they attempt to replenish their nutritional reserves. On the other hand, if a female game birds' nutritional needs are satisfied, egg laying will continue throughout the breeding season. Identification of nutrients which effect the breeding condition of a female game bird would allow game bird breeders to supplement the diets of these birds and thus maximize the number of game birds produced during each breeding season.

A first step for identifying a role for important dietary nutrients in game birds would be empirical evidence of a reproductive effect. Reproduction in New World Quail has been linked to an unknown factor present in vegetation consumed by breeding adults (Leopold, 1977). Game birds are primarily herbivorous and increase the intake of green vegetation prior to, and during the breeding season (Leopold, 1977; Gutiérrez, 1980; Johnsgard, 1988). Game bird species throughout North America, Europe, Asia and Africa breed seasonally and lay large clutches suggesting a periodic increase in

consumption of dietary nutrients during egg formation (Johnsgard, 1988). The breeding ranges of these species include arid regions with variable precipitation and subsequent variations in the amount of vegetation available for consumption. In these areas, game birds such as wild quail demonstrate high fecundity during moist years when large amounts of green vegetation are available for consumption, and low fecundity during dry years when vegetation is scarce (Leopold, 1977).

2. Xanthophyll.

Several attempts have been made to identify dietary nutrients available from green vegetation which contributes to the reproduction rates in New World Quail (Fletcher, 1971; Leopold, 1977; Cain *et al.*, 1987). Early studies focused on Vitamin A in Northern Bobwhites (*Colinus virginianus texanus*) (Lehman, 1953) and Gambel's Quail (*Callipepla gambelii*) (Hungerford, 1964), while later studies examined the possible role of dietary phytohormones in California Quail (*Callipepla californica*) (Leopold, 1977) and phytoestrogens in Scaled Quail (*Callipepla squamata*) (Cain et al., 1987). These studies failed to identify a dietary factor promoting fecundity in game birds.

Xanthophyll is a yellow to red carotenoid pigment found in photosynthetic plants and vegetation and is conspicuously concentrated in the follicles of egg-laying species, producing a characteristic yellow color to avian and reptile egg yolks (Burley and Vadehra, 1989) and various hues of yellow to red in the eggs of fish, amphibians and insects. Xanthophyll is a member of the class of oxygenated carotenoid pigments and has been used as a component in poultry feed for many years as a means of enhancing the yellow color of egg yolks and skin pigmentation (see U.S. Patent 3,020,159; Fry and Harms, 1975; Narahari, 1981; Papa *et al.*, 1985; Ouart *et al.*, 1988; Hencken, 1992). Xanthophyll is also sequestered in the reproductive tissue of most higher vertebrates during reproduction (Goodwin, 1950). The sequestration of xanthophyll in reproductive organs suggests that this carotenoid plays an important role in reproduction across species. More recently, one type of xanthophyll known as astaxanthin, has been identified as an agent which enhances fecundity in commercial poultry (see U.S. Patent

5,744,502; WO9608977).

Astaxanthin was also incorporated into a commercial feed for pregnant sows as described in PCT application PCT/SE97/00488. The data indicates a litter weight increase and weaning to remating interval decrease (see WO9735491).

The mechanism of action by which xanthophyll exerts its effect on reproductive capability is not known but has previously been assumed to be related to its antioxidant properties (see U.S. Patent 5,744,502). However, these assumptions do not account for the fact that steroid-mediated functions predominate in tissues where xanthophyll accumulates and that steroid hormones directly regulate gene expression.

3. Effects of Xanthophyll on Estrogen-mediated Processes.

The regulation of gene expression by steroid hormones is common to all vertebrate animal species and is a result of the fundamental mechanism of action of steroid hormones. Steroid molecules regulate gene expression by binding to specific receptor proteins in the nucleus of a cell which in turn bind directly to DNA or DNA accessory proteins leading to transcription of the DNA coding for any particular gene. Identification of factors which alter the effect of steroid hormones would therefore be useful in modulating the effect of any particular steroid hormone.

Environmental estrogens and estrogen mimetics that interact with steroid-mediated processes can negatively affect human and wildlife health and reproduction (Hileman, 1994; McLachlan and Arnold, 1996). The incidence of human diseases in steroid sensitive tissues in the United States has increased in recent years and has been tentatively linked to diet and exposure to other environmental factors (Colburn *et al.*, 1996). This has occurred during a period when dietary consumption of concentrated xanthophyll by humans has increased significantly due to artificial bio-accumulation in food such as poultry flesh and egg yolks. It has been observed that circulating levels of xanthophyll in humans have been positively correlated to dietary intake (Campbell *et al.*, 1994; Brady *et al.*, 1996; Scott et al., 1996).

SUMMARY OF THE INVENTION

The present inventor has discovered that xanthophyll can enhance or accentuate the effects of steroids, particularly estrogen, on physiological processes in animals. The effect that xanthophyll can enhance include estrogen-mediated events in reproduction.

Thus, one aspect of the present invention relates to a method of increasing the reproductive capability of a female game bird by administering xanthophyll to the game bird, particularly by supplementing the diet of the game bird with xanthophyll. A related aspect of the invention involves methods to accelerate annual recrudescence of the oviduct and ovary in a female game bird by supplementing the diet of the game bird with xanthophyll. Another aspect of the invention involves a method of enhancing steroid-mediated reproductive responses in a game bird by supplementing the diet of the game bird with xanthophyll.

Preferred dosages of xanthophyll involve feeding the game bird at least about 1 milligram of xanthophyll per day. The xanthophyll may be derived from a variety of sources, including extracts of marigold flowers, alfalfa, corn, algae, lucerne, Sudan grass and other grasses, red pepper, paprika, fruit, flower petals and fungi. A variety of xanthophylls may be used alone or in combination, and include free xanthophylls, esterified xanthophylls, zeaxanthin, astaxanthin, canthaxanthin, capsanthin, antheraxanthin, and cryptoxanthin.

The methods, and feed (or chow) formulations of the present invention may be utilized with a variety of game birds, such as New World quails (*Odontophoridae*), partridges and francolins (*Phasianidae* and *Perdicinae*), pheasants (*Phasianidae* and *Phasianinae*), grouse (*Tetraonidae*), wild turkeys (*Meleagrididae*), guineafowl (*Numidae*), waterfowl (*Anseriformes*), megapodes (*Megapodidae*), Cracids (*Cracidae*), ostriches (*Struthionidae*), rheas (*Rheidae*), doves and pigeons (*Columbiformes*). A preferred game bird chow composition would contain at least about 1 milligram of xanthophyll per kilogram of chow. Other contemplated formulations would contain at least about 24 milligrams or more, such as at least about 100 milligrams of xanthophyll per kilogram of chow.

In concentrations higher than the xanthophyll concentrations of conventional

poultry chows, another aspect of the present invention relates to methods and chows useful in accentuating the effects of estrogens in domesticated poultry such as chicken and turkeys.

In yet another aspect, the present invention relates to methods where the feeding or administration of xanthophyll produces one or more effects selected from the group consisting of: (1) accelerating the onset of breeding capability; (2) increasing time that a game bird remains in breeding condition; (3) increasing fecundity in a female game bird; (4) enhancing sexual maturation; and (5) promoting sexual behavior.

Chow compositions are particularly contemplated as being prepared with packaging materials and a label associated with the packaging that indicates that the chow appropriately would be fed to game birds or to poultry. Methods of preparing game bird chow formulations according to the present invention also are contemplated.

Another aspect of the present invention relates to methods for accentuating the effects of estrogen in an animal, particularly a mammal, by administering an effective amount of xanthophyll. Such effects include coming into estrus, engaging in sexual reproductive behaviors, ovulating, forming a corpus luteum, undergoing uterine and mammary development preparatory to pregnancy and parturition. A preferred application of the present invention would be administer the xanthophyll in an amount that is effective to accentuate the physiological effects of estrogen in menopausal or post-menopausal females.

In a related aspect, the present invention includes methods of enhancing steroid-mediated reproductive responses in an animal, particularly a mammal, by supplementing the diet of the mammal with an effective amount of xanthophyll. Such responses include delaying uterine atrophy, maintaining uterine cervical integrity, prolonging the regular renewal of breast tissue and delaying bone demineralization.

In a composition or formulation aspect, the present invention also relates to an estrogen replacement composition comprising an amount of xanthophyll effective to accentuate the effects of estrogen, preferably in a diet supplement or in a unit dose form. Such compositions may be co-formulated or co-administered with antioxidants or other estrogen replacement or estrogen supplementation compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows mean and standard error of oviduct mass of yearling female mountain quail supplemented with dietary xanthophyll for two weeks. Values are expressed as percent of total body mass. Numbers above error bars indicate sample size.

Figure 2 shows mean and standard error female mountain quail fecundity as measured by daily egg production per female within breeding pens provisioned with control or xanthophyll-supplemented food. Xanthophyll (n=six) and control (n=six) pens housed equal numbers of females. Total egg production was measured 14 days after beginning xanthophyll supplements and at the end of the breeding season.

DETAILED DESCRIPTION OF THE INVENTION

I. General.

The present invention is based on observations that animals sequester dietary carotenoids, including xanthophyll, into tissues that require the action of steroid hormones for their growth, development, or performance. These tissues include oviducts, ovaries, seminal vesicles, adrenal glands, corpora lutea of pregnant mammals, skin, feather, and many other tissues. The action of steroid hormones in these tissues, in turn, requires the presence of metabolic breakdown products of carotenoid pigments. For example, carotenoid pigments are split and modified to form retinoic acids (RA). For steroid hormones to work, four molecules must be present: (1) a retinoic compound such as RA, (2) a receptor molecule for the retinoic compound, (3) a steroid hormone, and (4) a receptor molecule for the steroid hormone. The metabolic breakdown products of xanthophyll are oxygenated and are highly active biologically. The result is that when xanthophyll is present in these tissues (yielding oxygenated retinoic compounds), or the oxygenated retinoic metabolites themselves are present, the performance of the steroid hormones is enhanced and subsequently the performance of the steroid-sensitive tissue is enhanced. By making xanthophyll available to the animal, steroid-mediated events, particularly estrogenic events, are enhanced.

Using quail and pheasants as study organisms, the present inventor has demonstrated that estrogen-sensitive tissues grow and perform at an accelerated rate when the diet of the animal is supplemented with xanthophyll. For example, the oviducts of female quail require elevated levels of the steroid hormone estrogen in order for the oviducts to grow and become functional. The quail is infertile and sexually immature until the oviduct is fully formed. When quail are supplemented with dietary xanthophyll, the oviducts of female quail grow and become functional more quickly, making the bird fertile and ready to breed and produce eggs earlier in the breeding season.

This discovery has wide-ranging implications in that it provides a means by which estrogen performance can be modulated used natural dietary supplements. For example, in animal husbandry, it provides a means for bringing animals into breeding condition faster without the use of administered hormones. In humans, it may provide a means for enhancing the performance of estrogen as an alternative to taking estrogen (or other steroid) supplements.

II. Definitions.

"Accentuating the effects of estrogen in an animal" means causing a more pronounced response or response of greater magnitude of the physiological event or the behavior mediated by estrogen in the animal, such as game birds and mammals, including human beings.

"Annual recrudescence of the oviduct and ovary" means the annual regrowth of these organs in game birds.

"Breeding capability" means ability to engage in reproductive sexual behavior, ability to form and deliver mature, functional gametes (eggs or sperm), ability to form viable offspring, and ability to carry out physiological events necessary for reproduction (such as maintaining pregnancy and giving birth).

"Breeding condition" means being in any of a series of physiological states related to successful reproduction beginning with the organism able to form and deliver mature gametes through the creation of viable offspring and ending when the organism is no longer capable of reproduction without a substantial change in its physiological status.

"Chow" means a commercial formulation of nutritive components formulated for particular species or health conditions

"Effective amount" means the amount, for example, of xanthophyll that is effective to accentuate the effects of estrogen in an animal or to enhance a desired physiological effect of estrogen, such breeding capability, reproductive capability or steroid-mediated reproductive response, compared with the level of such physiological effect in the absence the administration of xanthophyll. For example, in experiments with quail, approximately 2.0×10^{-5} grams of xanthophyll per gram of body weight per day were consumed. One skilled in the art would understand that food consumption depends on many things such as species, body size, and ambient temperature, and that gut absorption rates of xanthophyll vary among species. It is contemplated that much lower doses also would be effective in quail, such as about 2.0×10^{-7} grams of xanthophyll per gram of body weight per day. This corresponds, for game birds, to supplementing the diet at the rate of about 2.4 mg xanthophyll / kg chow.

In general, for administration to animals, including mammals and human beings, a range of about 2.0×10^{-7} grams of xanthophyll per gram of body weight per day to about 2.0×10^{-5} grams of xanthophyll per gram of body weight per day is contemplated. One skilled can readily vary or optimize these dosages, particularly in light of the scientific literature which shows levels of xanthophyll compounds that have biological effect, for example, as anti-tumor agents. See, for example, the effective dosages reported in the literature, such as Dietary lutein from marigold extract inhibits mammary tumor development in BALB/c mice. J Nutr 1998 Oct; 128 (10): 1650-6; and E.P. Chew and M.W. Wong and P.S. Wong (1996) Anticancer Research 16:3689. See, also, D. Kostic and W. S. White and J. A. Olson (1995) American Journal of Clinical Nutrition. 62:604 who reported that 0.5 muMol per kg/body weight of lutein in true solution in oil elevates circulating levels of lutein. Relevant scientific articles also describe the xanthophyll content of certain foods that show biological effect. See, for example, M. Micozzi *et al.,* Carotenoid Analyses of Selected Raw and Cooked Foods Associated With a Lower Risk for Cancer, J Natl Cancer Inst 1990; 82: 282-285 (1990).

"Game bird" means birds species traditionally and/or currently subject to hunting

for sport, plumage, or subsistence. Examples of game birds include, but are not limited to, New World quails (*Odontophoridae*), partridges and francolins (*Phasianidae* and *Perdicinae*), pheasants (*Phasianidae* and *Phasianinae*), grouse (*Tetraonidae*), wild turkeys (*Meleagrididae*), guineafowl (*Numidae*), waterfowl (*Anseriformes*), megapodes (*Megapodidae*), Cracids (*Cracidae*), ostriches (*Struthionidae*), rheas (*Rheidae*), doves and pigeons (*Columbiformes*). The term "game bird" is intended to exclude poultry such as chicken and domestic turkey that are commercially bred for consumption or egg production. It is contemplated that the methods and compositions of the present invention are applicable to birds, such as ostriches and rheas, that are traditional game birds in Africa and South America, respectively, but which are now being raised in captivity for meat production in the US and Europe. As contemplated herein, they are not "commercial poultry" even though they are raised for meat rather than, for example, for release to hunting clubs.

"Packaging" means the containers used to store or transport feed or chow, including cartons, bags, wrapping and other conventional devices uses for such purposes.

"Reproductive capability" means the degree to which an animal is capable of producing functional gametes and ultimately viable offspring.

"Steroid-mediated reproductive response" means physiological and/or behavioral events relating to reproduction the occurrence of which is determined by the prevailing levels of specific steroid hormones. For example, oviduct recrudescence in birds is mediated by the level of circulating estrogen; intramale aggression in deer is mediated by level of circulating testosterone and so on.

"Xanthophyll" means the class of molecules consisting of several hundred types of oxygenated carotenoid pigments. The term xanthophyll includes the specific forms lutein, zeaxanthin, capsanthin, astaxanthin, and canthaxanthin but is not limited to these forms. The term "free xanthophyll" means the xanthophyll molecules occur in their pure state, not esterified to lipids or chemically bound to proteins or other molecules.

III. Specific Embodiments.

A. Methods of enhancing reproductive capability in game birds.

The present invention provides methods for enhancing reproductive capability in birds, particularly game birds. Game birds differ from domestic poultry in many important ways. One especially important difference is that game birds breed seasonally rather than year-round. Due to complex genetic reasons, when animals are domesticated, such as domestic poultry, they lose the strong seasonality to breeding that characterizes wild animals. This is a critical issue in game bird breeding. A primary objective of most game bird breeders is to raise birds with wild characteristics for release into the wild. Inevitably, to raise game birds with highly desirable wild characteristics the game bird breeder uses breeding stock that only breeds seasonally. For most game birds this is a period of 7-8 weeks during the spring. Thus, game birds breeders have a short window of opportunity each year in which to produce their annual "crop" of game birds.

The problem of seasonal reproduction faced by game bird breeders is complicated by a second issue. Regardless of age, female game birds must grow ("recrudesce") their oviducts and ovaries anew each breeding season. In other words, before any egg production can occur, each spring a female game bird must increase the size of her oviduct and ovary several hundred to several thousandfold. (These sexual tissues regress following each breeding season.) By providing game bird breeders with a chow that reliably bring females into breeding condition and maximizes length of the breeding season game birds breeders would be able to operate with increased efficiency and output.

The present invention thus relates to game bird chows that brings female game birds into breeding condition sooner by promoting the rapid growth of the oviduct and ovary (a steroid dependent process) in the spring when estrogen levels in females begin to increase. Such chow formulations also extend the breeding season by keeping the oviduct and ovary functional longer during the waning period of the breeding season. Lastly, such chows result in a higher rate of egg production following the onset of the breeding season. Thus, the chow supplements and chow formulations according to the present invention provide an answer to the problem of the seasonality of breeding for game bird breeders.

B. General Methods of Preparing Game Bird Chow.

The game bird feed compositions contemplated for the present invention generally provide at least about 1 milligram, more preferably at least about 5, 10, 20, 30, 40 or 50 milligrams of xanthophyll per kilogram of feed composition. By way of example, Purina Gamebird Breeder Layena, is preferred as a base chow formulation for supplementation with xanthophyll. However, the particular chow utilized is not critical for the purposes of the present invention.

Although feed compositions for poultry, such as chicken and domestic turkey, are known to include xanthophyll, before the present invention, feed compositions intended for the diet of game birds were not so formulated with xanthophylls at a level to be effective for accentuating the effects of estrogen as contemplated herein. In some embodiments of the present invention, higher levels of xanthophyll may be used, such as at least about 75, 100, 150, 200 or 250 milligrams of xanthophyll per kilogram of feed composition.

In one embodiment, an avian chow was supplemented with 109 mg of the carotenoid pigment xanthophyll per one pound of basic avian chow. This was accomplished by mixing 454 g of the commercially available xanthophyll supplement, CHROMOPHYLL-ORO™ with 50 lbs. of commercially available PURINA GAMEBIRD LAYENA™ or PURINA FLIGHT CONDITIONER™. The xanthophyll supplement and 50-lbs. avian chow were placed in an airtight container, which was sealed and rolled at room temperature until the two compounds were thoroughly mixed. The xanthophyll supplement evenly coats the individual particles of avian chow. When the resulting mixture is fed to birds, as birds eat the chow they also consume the xanthophyll-bearing outer coating. This leads to a pronounced supplement of xanthophyll in the diets of birds, exceeding that historically included in commercial game bird chows.

The precise dose of xanthophyll supplemented or fortified chow according to the present invention can be varied by the skilled artisan in order to accomplish various objectives. For example, to promote sexual maturation and egg laying in birds generally, a preferred chow formulation comprises a standard chow supplemented with 1 or more

mg xanthophyll per pound (or per kilogram) chow. For commercial chickens reared to lay eggs for human consumption, i.e., "layers," an appropriate formulation comprises avian chow supplemented with xanthophyll at a dose greater than about 26 mg of xanthophyll per kilogram of chow. In fact doses or concentrations greater than about 30, 40, 50, 60, 75 or 100 mg per kilogram of chow are contemplated. The skilled artisan will realize that current commercial chows designed for layers may already incorporate xanthophyll at a doses up to 26 mg/lb. chow mixes for the purpose of ensuring a golden color to the yolks of eggs sold for human consumption. However, it has not been appreciated that chows formulated according to the present invention would have additional beneficial effects with respect to the use of xanthophyll supplements to laying hen performance.

Formulations of chow according to the present invention are designed to increase rate of sexual maturation and fecundity in birds generally including quails, partridges, pheasants, grouse, turkeys, guineafowl, francolins and other galliforms, anseriforms, ratites and ostriches. Such products also are intended for chicken hens used to produce fertile eggs for hatching (*i.e.*, chick production operations). At xanthophyll doses greater than 26-mg/lb. chow, such formulations would be intended for layers (*i.e.*, to enhance egg production).

C. The Role of Xanthophyll in Steroid Activity

Animals (including humans) consume dietary xanthophyll and sequester high concentrations of it into the tissues of reproduction and tissues that produce steroid hormones. Xanthophyll is metabolized to form retinoic compounds within animals. As noted above, retinoic compounds are central to the performance of steroid hormone receptors by enabling the hormone receptors. Active steroid hormone receptors, in turn, are necessary for the steroid hormones to work. Thus, dietary xanthophyll promotes steroid performance in animals (including humans) by its own action and by providing key metabolites that are necessary for steroid hormones to carry out their many functions.

Estrogen is a steroid hormone, and disruption of its performance causes of human

and wildlife diseases. The sensitivity of estrogen receptors to dietary components and other compounds is particularly well understood due to the intense investigation of compounds that disrupt estrogen receptors. Such compounds may be consumed in the diet, and include, for example, DDT, DDE, dieldrin, endosulfan, toxaphene, chlordane, and other pesticides, PCBs, and certain detergents.

The game birds described in the examples that follow will be recognized by those skilled in the art as being appropriate animal models to validate the finding that dietary xanthophyll accentuates the physiological effects of estrogen. This is because the way that estrogen and its receptor systems exert their effects is common among birds, mammals (including humans), and other animals. In fact, for example, a great many wild birds and mammals are known to switch to diets high in carotenoids prior to breeding. A. S. Leopold, *The California Quail*. University of California Press, Berkeley (1977). Thus, a person skilled in the art would understand that the estrogenic effect of dietary xanthophyll demonstrated below with respect to game birds would be reasonably predictive of the estrogenic response in other animals.

Retinoic acids, which result from metabolizing dietary xanthophyll, are a critical participant in allowing the action of steroid hormones to occur. This is true for species throughout the animal kingdom. See, for example, Thomas, H.E., Stunnenburg H. G., and Stewart A. F. 1993. Heterodimerization of the Drosophila ecdysone receptor with retinoid-X receptor and ultraspiracle. Nature 362: 471. Retinoic acids, as result from metabolizing dietary xanthophyll, affect the performance of estrogen and other steroid hormones in humans and other animals by interacting with the intracellular protein receptors of steroid hormones. The mechanism of action of steroid hormones involves their binding to receptor proteins within the cell. Estrogen receptors share a high degree of similarity across animal taxa. Weida, T. et al. 1997. Quantitative structure-activity relationships (QSARs) for estrogen binding to the estrogen receptor: Predictions across species. Environmental Health Perspectives 105: 1116.

In turn, retinoic acids enable the steroid hormone-by-receptor complex to activate target genes within the DNA complex of the cell. See, Means, A. L and Gudas L. J. 1995. The roles of retinoids in vertebrate development. Annu. Rev. Biochem. 64: 201;

and Thomas, cited above. In other words, the presence of retinoic acids, as results from dietary intake of xanthophyll, promotes the action of steroid hormones in humans and other animals. Thus, the positive effect of xanthophylls and retinoic acids on steroid performance in animals includes the enhanced activity of estrogen and estrogen receptors.

In humans, birds, and other animals, the level of xanthophyll in circulation within the body is positively correlated to dietary intake and lifestyle factors. See, Scott, K. J., D. I. Thurnham, D. J. Hart, S. A. Bingham, and K. Day. 1996. The correlation between the intake of lutein, lycopene and beta-carotene from vegetable and fruits, and blood plasma concentrations in a group of women aged 50-65 years in the UK has been demonstrated. Brit. J. Nutr. 75: 409; Campbell, D. R., M. D. Gross, M. C. Martini, G. A. Grandits, J. L. Slavin, and J. D. Potter. Plasma carotenoids as biomarkers of vegetable and fruit intake. Cancer Epidemiology, Biomarkers & Prevention 3: 493; Hill, G. E. 1996. Redness as a measure of the production cost of ornamental coloration. Ethology, Ecology, and Evolution 8: 157.

Overwhelming evidence from the field of ecotoxicology indicates that biologically active compounds that affect estrogen receptor performance and estrogen activity work across a wide range of animal taxa, including humans, such as the pesticides noted above. For example, exogenous compounds consumed in the diet affect endogenous estrogen function in humans. Colburn, T. 1994. The wildlife/human connection: modernizing risk decisions. Environmental Health Perspectives 102 (Suppl 12): 55. The action of estrogen receptor disrupters negatively affects humans, other mammals, birds, fish, shellfish, and gastropods. Colburn, T, vom Saal, F. S., and Soto, A. M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives 101: 378.

A close relationship exists between compounds affecting steroid hormone receptors and human diseases or syndromes of steroid-sensitive tissues. These include: cancers of the breast, ovary, endometrium, testes, prostate, thyroid as well as the syndromes of hypospadius, cryptoorchidsism, reduced size of sex organs, reduced sperm count, reduced sperm production, reduced semen volume, and reduced fecundity.

Gillesby, B. E., and T. R. Zacharewski. 1998. Exoestrogens: Mechanisms of action and strategies for identification and assessment. Environmental Toxicology and Chemistry 17: 3.

The corpus luteum which forms within the ovary of pregnant humans and many other live-bearing animals contains a very high concentration of xanthophyll. Porter, J. W. and Spurgeon, S. L (eds). 1983. Biosynthesis of Isoprenoid Compounds. John Wiley and Sons, New York. The corpus luteum is both sensitive to and produces steroid hormones and is essential to regulating appropriate uterine growth relative to the state of pregnancy.

Retinoic acids, as result from metabolizing xanthophyll, also are critical to proper embryological development, which is a steroid-mediated growth process. J. A. Olson, and N. I. Krinsky, *FASEB J.* **9**, 1547 (1995); and R. S. Parker, *FASEB J.* **10**, 542 (1996). For example, proper limb and body axis development depends on presence of retinoic acids. For reproduction to be successful in egg-laying species, dietary carotenoids like xanthophyll or their metabolites must be present within the breeding female in order to be deposited into eggs during egg-formation. In live-bearing organisms like humans, dietary carotenoids like xanthophyll or their metabolites must be present in circulation prior to and during pregnancy. Steroid receptors are targets for several important drugs in humans. Reduced levels of circulating xanthophyll is associated with advancing stages of cervical cancer in humans. Potischman, N. et al. 1994. The relations between cervical cancer and serological markers of nutritional status. Nutrition and cancer 21: 193.

D. Applications of Xanthophyll to Accentuate Estrogen Effects.

Several uses of xanthophyll according to the methods of the present invention are contemplated. For example, dietary xanthophylls may be administered or consumed in order to enhance (increase) human female breast size. The growth and maintenance of human breast tissue is mediated by estrogen. Increasing the performance of endogenous estrogen should promote growth and maintenance of breast tissue. In this regard, certain "herbal" treatments are available for which their component grains and herbs are asserted

to have natural estrogenic properties that interact with estrogen receptors. An alternative product is a dietary supplement with a safe and known dose of xanthophylls designed to enhance estrogen effects.

Dietary xanthophyll supplements also could replace or augment carotene vitamin supplements such as beta carotene. Where the combined effects of an antioxidant and an estrogen enhancer are desired, as would be the case for women taking vitamin supplements as a defense against breast cancer, xanthophyll supplements would be superior to beta-carotene. Xanthophyll also could be administered or consumed with one or more other estrogen enhancers, optionally together with an antioxidant compound. One or more xanthophyll compounds also may be administered rather than a single xanthophyll compound. For such purposes, xanthophyll can also be co-formulated with one or more such compounds or co-administered either simultaneously or in a common therapeutic window.

An additional contemplated use would be the administration of controlled levels of dietary xanthophylls to treat infertility syndromes that are due to low estrogen levels or poor estrogen performance in humans.

From an animal breeding perspective, the compositions and methods of the present invention may be used to manipulate dietary xanthophyll to bring valuable birds in the pet industry and zoological gardens into breeding condition. For example, parrots are bred commercially for the pet trade and endangered parrot species are bred in captivity for conservation reasons. Individual parrots can be both rare and expensive. The ability to regulate their reproductive patterns would have commercial value and public benefit.

Blood levels of xanthophyll may be measured and monitored by various techniques known to those skilled in the art. Such techniques are described, for example, in G. Rumi *et al.*, Decrease in serum levels of vitamin A and zeaxanthin in patients with colorectal polyp. Eur J Gastroenterol Hepatol 1999 Mar; 11 (3): 305-8; and R. Parker, Carotenoids in human blood and tissues. J Nutr 1989 Jan; 119 (1): 101-4.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure. All articles, publications, patents and documents referred to throughout this application are hereby incorporated by reference in their entirety.

EXAMPLES

Example 1: Effects of xanthophyll on Estrogen-mediated physiological processes in game birds.

The effect of dietary xanthophyll on reproductive tissue recrudescence in breeding mountain quail was examined as a means for assessing the effect of xanthophyll on Estrogen-mediated physiological processes. First, the availability of green plants and yellow grains in quail diets beyond that available in standard commercial grain-based chows was minimized as to control the dietary intake of xanthophyll. Second, the diets of experimental quail during the breeding season were supplemented with a commercially available poultry diet xanthophyll-supplement.

Like other seasonally breeding birds, mountain quail females recrudesce their ovary and oviduct, and males their testes, each spring. Forty-six yearling quail entering their first breeding season were used to test the effect of dietary xanthophyll on reproductive tissue recrudescence. Thus, all quail were undergoing their first reproductive tissue recrudescence. Juvenile quail were housed outdoors on gravel through winter without access to green vegetation and fed *ad libitum* a complete ration commercial game bird maintenance chow (Purina Gamebird Flight Conditioner). In April, two males and two females were placed in each of twenty-three 2.44 x 2.44 meter outdoor breeding pens. Two males only were placed in a twenty-fourth pen. Breeding pens were maintained free of growing green vegetation. Pen floors consisted of a ten centimeters mixture of unsorted, fine gravel high in clay minerals and three millimeter granite chips. These were underlain with plastic sheeting to prevent green plant growth.

Each pen was equipped with lean-to shelters, "brush" in the form of small dry conifers cut five months previously, pine logs, and dried pine needles under lean-tos and brush to serve as nesting material.

Twelve pens were provisioned *ad libitum* with a control diet consisting of a complete ration game bird laying chow (Purina Game bird Layena). Precise grain mixes making up commercial chows vary dependent on grain availability and market prices. Visual inspection indicated that yellow corn was not a prominent component in the Game bird Layena used in these feeding trials. Twelve pens were provisioned with a treated diet. In treated pens, Purina Game bird Layena was supplemented with xanthophyll at a dose of 240 milligrams xanthophyll per kilogram laying chow. The lipid, carbohydrate, and mineral changes in quail diets caused by the addition of Chromophyl-Oro was trivial. The xanthophyll supplement increased lipids by a maximum of 0.2% and decreased protein and ash by a maximum of 0.2% and 0.1%, respectively. After 14 days in breeding pens, one random male and female were collected from each pen (both males from the 24th pen) and frozen until analyzed.

Quail were thawed, weighed and several morphometric measures taken before dissection. The length of longest head plume, culmen, nalopsi, head width posterior to the orbits, wing chord, longest primary, central retrices, and chord of the central claw of the right foot was measured. During dissection, the breast of each quail was measure along with the sternum chord as the inner length from the base of the furculum to the tip of the xiphoid process of the sternum. For females, the diameter of the largest ovum within the *in situ* ovary was measure using a hand-held caliper. The ovary was then removed and weighed. The entire oviduct from the infundibulum to the vaginal opening of the cloaca was also separately weighed. For males, the length and width of each testes was measured *in situ*, after removal the weight of each testes was also determined.

Estrogen-mediated physiological processes were significantly amplified in female mountain quail receiving xanthophyll supplements. Yearling females rapidly enlarged their reproductive tracts in response to CHROMOPHYL-ORO™, a commercially available dietary xanthophyll supplement widely used in poultry and egg production in the United States, in their diets. CHROMOPHYL-ORO™ consists of highly

concentrated lutein as confirmed by HPLC analysis.

After two weeks on a xanthophyll-supplemented diet, yearling females had significantly larger oviducts ($Z = 2.24$, $p = 0.025$) than did control females (Figure 1). This included several females with well developed shell glands and oviduct musculature. Maturation of ova was accelerated among supplemented yearling females, as well. Breadth of largest ovum within the ovary was significantly greater among supplemented females ($Z = 2.14$, $p = 0.032$). Ova maturation led to a strong trend for greater ovarian mass among xanthophyll-supplemented females ($Z = 1.65$, $p = 0.099$; Figure 2).

Example 2: Effect of xanthophyll on reproduction in game birds.

The effects of dietary xanthophyll on egg-laying patterns were tested in a manner similar to tests of tissue recrudescence, but they were performed during a separate breeding season using different mountain quail. Quail were housed outdoors on gravel as a communal flock in a single large pen from October through February. In late winter, prior to observing any copulations, quail were segregated by sex into partitions within a large pen. Quail were fed Purina Game Bird Flight Conditioner *ad libitum* as their sole ration throughout this period. During early spring, three quail were placed in each of twelve 4.88 x 2.44 meter breeding pens. Males were added to breeding pens first. Females were then added to breeding pens one day after observing the first egg produced within the all female winter flock. Each trio of quail consisted of two females and one male or two males and one female in a balanced randomized design. Breeding pens were provisioned *ad libitum* with either unaltered Purina Game Bird Flight Conditioner ($n = 6$) or Flight Conditioner supplemented with xanthophyll at a ratio of 240 milligrams xanthophyll per kilogram Flight Conditioner ($n = 6$).

After being introduced into the breeding pens, quail quickly built nests and began laying eggs in the nests. Two weeks after females were added, the total number of eggs laid within each breeding pen was determined and the number of eggs laid per female per pen was calculated. Eggs were not removed from their nests. At this time, one quail from each trio was removed from each pen, leaving one bird of each sex. Remaining females were allowed to continue to lay until reproductive behavior had ceased for the

season. The total number of eggs laid was again determined and the number of eggs laid per female per pen per female-day was calculated. Female-day, consisting of total number of days each female was present in a given pen, adjusted for the fact that three pens in each treatment group had two females present for the first 14 days of the experiment.

The effect of supplementing diets with Chromophyl-Oro extended to egg-laying in both rate of laying and total numbers of eggs laid. Xanthophyll-supplemented females laid significantly more eggs than did control females during the first two weeks on experimental diets ($Z = 2.40$, $p = 0.016$; Figure 4). Xanthophyll-supplemented females also laid more eggs throughout the breeding season than did control females ($Z = 1.93$, $p = 0.054$; Figure 5).

Example 3: Production of game bird chow supplemented with xanthophyll.

This was achieved by mixing commercially available poultry ration pigment supplement Chromophyl-Oro (Chemtech Division of Girsa, Houston, TX) to Purina Game bird Breeder Layena. This product contains:

Crude protein no less than	20.0%
Crude fat not less than	2.5%
Crude fiber not more than	7.0%
Calcium (Ca) not less than	2.3%
Calcium (Ca) not more than	3.3%
Phosphorus (P) not less than	0.8%
Salt (NaCl) not less than	0.4%
Salt (NaCl) not more than	1.0%
Ash not more than	10.5%
Added mineral not more than	5.0%

Ingredients listed on the packaging material are: Milo, wheat midlings, soybean meal, safflower, meat meal, canola, calcium carbonate, dicalcium phosphate, corn, salt,

L-lysine, choline chloride, pyridoxine hydrochloride, thiamine, biotin, calcium pantothenate, riboflavin supplement, niacin supplement, vitamin E supplement, vitamin A supplement, folic acid, vitamin B-12 supplement, menadione dimethylpyrimidinol bisulfate (source of vitamin K), DL-methionine, vitamin D-3 supplement, mangarous oxide, zinc oxide, copper sulfate, calcium iodate, sodium selenite.

Chromophyl-Oro, which is a preferred source of xanthophylls, is an extract of marigold flowers diluted with marigold petal meal, with 100% nutritional value in and of itself, for use as an additive in poultry feed to ensure a golden-yellow skin color in broilers or a natural deep yolk color in eggs. Additionally, manufacturer specification sheets described Chromophyl-Oro chemical properties as 95% "free xanthophylls" (minimum) with the remaining 5% consisting of xanthophyll intermediates and xanthophyll esters.

Game bird chow supplemented with xanthophyll consists of game bird chow supplemented with 109 milligrams of the carotenoid pigment xanthophyll per pound of game bird chow. This was accomplished by mixing 454 grams of the commercially available xanthophyll supplement, Chromophyl-Oro to 50 pounds of commercially available Purina Game bird Layena or Purina Flight Conditioner. The xanthophyll supplement and avian chow were placed in an airtight container, which was sealed and rolled at room temperature until the two compounds were thoroughly mixed. The xanthophyll supplement evenly coats the individual particles of avian chow. When the resulting mixture is fed to birds, as birds eat the chow they also consume the xanthophyll-bearing outer coating. This leads to a pronounced supplement of xanthophyll in the diets of birds, exceeding that historically included in commercial poultry or game bird chows.

Example 4: Modulation of estrogen-mediated physiological processes by xanthophyll in menopausal and post-menopausal women.

The impact of dietary xanthophyll on estrogen-mediated physiological processes in humans is similar to the results obtained by the administration of xanthophyll to other species. For example, dietary xanthophyll affects the levels and physiological effects of

estrogen in post-menopausal females. Factors such as age, body mass index (BMI), plasma and ovarian estrogen levels may be examined as previously described (Bancroft and Cawood, 1996). Women who are candidates for xanthophyll administration are categorized according to the following variables: menopausal status (based on menstrual history and pattern and plasma progesterone), age, BMI, oestradiol, oestrone, luteinizing hormone, follicle stimulating hormone, total estrogen and testosterone, androstenedione, free androgen index, dihydroepiandrosterone, dihydroepiandrosterone sulphate, cortisol and dietary xanthophyll intake. Xanthophyll is administered in the ranges discussed above, and alterations in circulating steroid levels are correlated with the clinical effects of dietary xanthophyll.

The administration of dietary xanthophyll, in addition to, in combination with or as an alternative to estrogen-replacement therapy in post-menopausal women, accentuates the effects of endogenous steroid hormones, particularly estrogen. Estrogen replacement or supplementation therapies are described, for example, in U.S. Patents No. 5,919,817; 5,908,638 and 5,891,868.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

REFERENCES

Bancroft J. and E.H. Cawood. Androgens and the menopause; a study of 40-60-year-old women. 1996. Clin. Endocrinol. 45:577-587.

Brady, W. E., J. A. Mares-Perlman, P. Bowen, and M. Stacewicz-Sapuntzakis. 1996. Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J. Nutr. 126:129-137.

Cain, J. R., R. J. Lien, and S. L. Beason. 1987. Phytoestrogen effects on reproductive performance of scaled quail. J. Wildl. Manage. 51:198-201.

Campbell, D. R., M. D. Gross, M. C. Martini, G. A. Grandits, J. L. Slavin, and J. D. Potter. Plasma carotenoids as biomarkers of vegetable and fruit intake. Cancer Epidemiology, Biomarkers & Prevention 3:493-500.

Colborn, T., D. Dumanoski, and J. P. Meyers. 1996. Our Stolen Future: Are We Threatening our Fertility, Intelligence, and Survival?: A Scientific Detective Story. Penguin Books, New York.

Fletcher, R. A. 1971. Effects of vitamin A deficiency on the pituitary-gonad axis of the California quail, Lophortyx californicus. J. Exp. Zool. 176:25-34.

Fry, J. L., and R. H. Harms. 1975. Yolk color, candled egg grade and xanthophyll availability from dietary natural pigmenting ingredients. Poult. Sci. 54:1094-1101.

Gutiérrez, R. J. 1980. Comparative ecology of the mountain and California quail in the Carmel Valley, California. Living Bird 18:71-93.

Hencken, H. 1992. Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. Poult. Sci. 71:711-717.

Hileman, B. 1994. Environmental estrogens linked to reproductive abnormalities, cancer. Chem. Eng. News 72(5):19-23.

Johnsgard, P. A. 1988. The Quails, Partridges, and Francolins of the World. Oxford University Press, U.K.

Lehman, V. W. 1953. Bobwhite population fluctuations and vitamin A. Trans. N. Am. Wildl. Conf. 18:199-246.

Leopold, A. S. 1977. The California Quail. University of California Press, Berkeley.

McLachlan, J. A., and S. F. Arnold. 1996. Environmental estrogens. Am. Sci. 84:452-461.

Narahari, D., K. Venugopal, A. G. Raj, P. Kothandaraman, and R. Kumararaj. 1981. Marigold flower meal as a source of xanthophyll for egg yolk pigmentation. Indiana J. Poult. Sci. 16:409-411.

Ouart, M. D., D. E. Bell, D. M. Janky, M. G. Dukes, and J. E. Marion. 1988. Influence of source and physical form of xanthophyll pigment on broiler pigmentation and performance. Poult. Sci. 67:544-548.

Papa, C. M., D. L. Fletcher, and H. R. Halloran. 1985. Utilization and yolk coloring capability of xanthophylls from synthetic and high xanthophyll concentrates. Poult. Sci. 64:1464-1469.

Scott, K. J., D. I. Thurnham, D. J. Hart, S. A. Bingham, and K. Day. 1996. The correlation between the intake of lutein, lycopene and beta-carotene from vegetable and fruits, and blood plasma concentrations in a group of women aged 50-65 years in the UK. Brit. J. Nutr. 75:409-418.

CLAIMS

1. A method of increasing the reproductive capability of a female game bird comprising the step of supplementing the diet of the game bird with xanthophyll.

2. A method of accelerating annual recrudescence of the oviduct and ovary in a female game bird comprising the step of supplementing the diet of the game bird with xanthophyll.

3. A method of enhancing steroid-mediated reproductive responses in a game bird comprising the step of supplementing the diet of the game bird with xanthophyll.

4. The method of any of claims 1, 2 or 3 wherein the game bird is fed at least about 1 milligram of xanthophyll per day.

5. The method of claim 4 wherein the xanthophyll is derived from a source selected from the group consisting of extracts of marigold flowers, alfalfa, corn, algae, lucerne, Sudan grass and other grasses, red pepper, paprika, fruit, flower petals and fungi.

6. The method of claims 4 wherein the xanthophyll is selected from the group consisting of free xanthophylls, esterified xanthophylls, zeaxanthin, astaxanthin, canthaxanthin, capsanthin, antheraxanthin, and cryptoxanthin.

7. The method of claims 1, 2 or 3 wherein the game bird is selected from the group comprising New World quails (*Odontophoridae*), partridges and francolins (*Phasianidae* and *Perdicinae*), pheasants (*Phasianidae* and *Phasianinae*), grouse (*Tetraonidae*), wild turkeys (*Meleagrididae*), guineafowl (*Numidae*), waterfowl (*Anseriformes*), megapodes (*Megapodidae*), Cracids (*Cracidae*), ostriches (*Struthionidae*), rheas (*Rheidae*), doves and pigeons (*Columbiformes*).

8. The method of claim 1 wherein the xanthophyll has at least one effect selected from the group consisting of: (1) accelerating the onset of breeding capability; (2) increasing time that a game bird remains in breeding condition; (3) increasing fecundity in a female game bird; (4) enhancing sexual maturation; and (5) promoting sexual behavior.

9. A game bird chow composition comprising at least about 1 milligram of xanthophyll per kilogram of chow.

10. The composition of claim 9, wherein the xanthophyll is selected from the group consisting of free xanthophylls, esterified xanthophylls, zeaxanthin, astaxanthin, canthaxanthin, capsanthin, antheraxanthin and cryptoxanthin.

11. The chow composition of claim 9, further comprising packaging and a label associated with the packaging that indicates that the chow appropriately would be fed to game birds.

12. The composition of claim 9, further comprising at least about 100 milligrams of xanthophyll per kilogram of chow.

13. A method of preparing a game bird chow comprising the steps of adding xanthophyll at a level of at least about one milligram of xanthophyll per kilogram of chow.

14. A method of increasing the reproductive capability of a female game bird comprising the step of supplementing the diet of the game bird with an effective amount of a xanthophyll.

15. A method of accentuating the effects of estrogen in a mammal comprising the step of administering an effective amount of xanthophyll.

16. The method of claim 15, wherein the effects of estrogen are selected from the group consisting of coming into estrus, engaging in sexual reproductive behaviors, ovulating, forming a corpus luteum, undergoing uterine and mammary development preparatory to pregnancy and parturition.

17. The method of claim 15, wherein the xanthophyll accentuates the physiological effects of estrogen in post-menopausal females.

18. A method of enhancing steroid-mediated reproductive responses in a mammal comprising the step of supplementing the diet of the mammal with an effective amount of xanthophyll.

19. The method of claim 17, wherein the reproductive responses are selected from the group consisting of delaying uterine atrophy, maintaining uterine cervical integrity, prolonging the regular renewal of breast tissue and delaying bone demineralization.

20. An estrogen replacement composition comprising an amount of xanthophyll effective to accentuate the effects of estrogen.

21. The composition of claim 20 in unit dose form.

FIG. 1

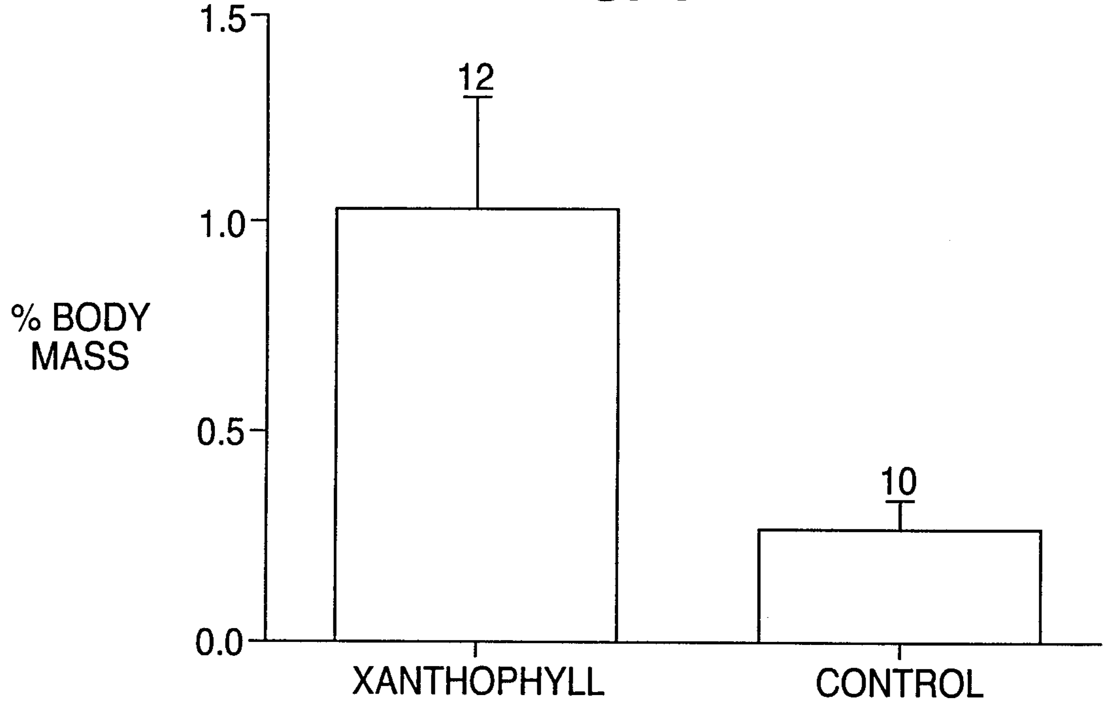
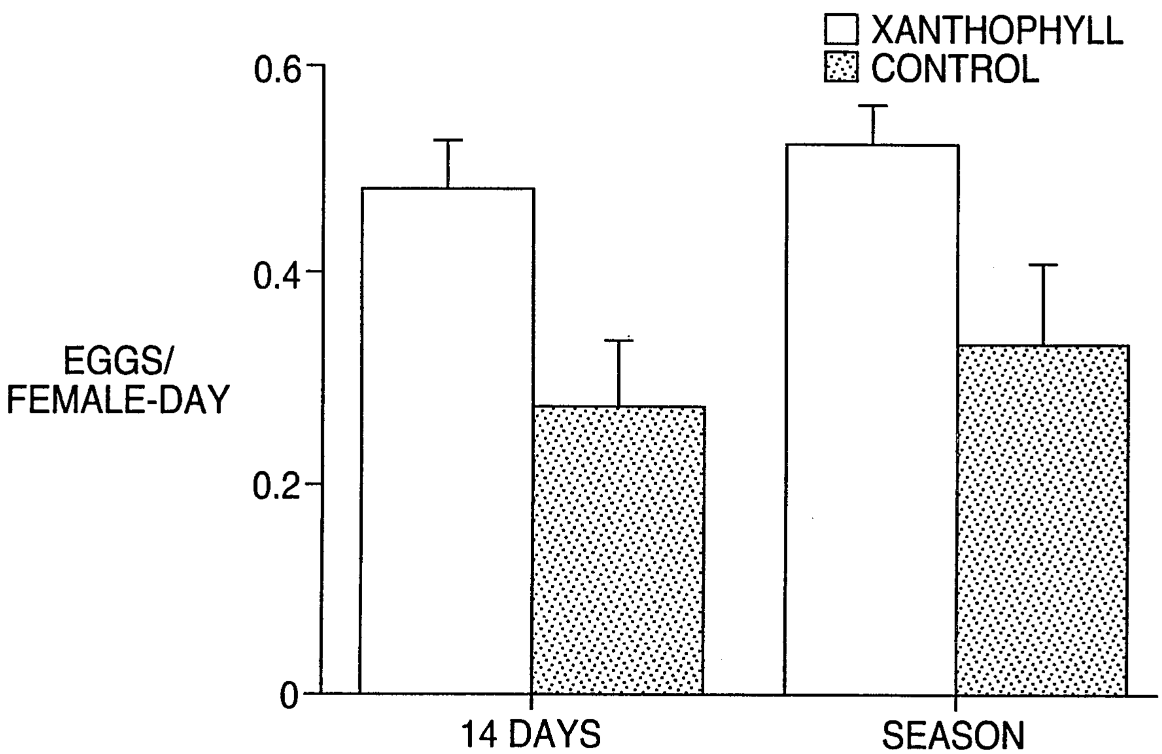


FIG. 2


XANTHOPHYLL
CONTROL
0.6
0.4
0.2
0
EGGS/
FEMALE-DAY
14 DAYS
SEASON

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17370

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/045
US CL :514/729
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/729

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

File BIOSCI- xanthophylls and estrogen or reproduction in birds and generally.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,744,502 A (LIGNELL et al.) 28 April 1998, see entire document.	9-10 and 20 ---------- 1-2, 4-8, 11-14, 21
X ---- Y	Derwent Information Ltd., File: DWPI, week 9922, AN 1997-489294, WO 9735491 A1 (INBORR et al.), abstract.	20 ---------- 1-2, 4-14, 21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 SEPTEMBER 1999

Date of mailing of the international search report

03 NOV 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

M. MOEZIE

Telephone No. (703) 308-1235